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Folate, methionine, alcohol, and colorectal cancer in a prospective study of women in the United States

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Abstract

Objective: We investigated the associations of folate, methionine, and alcohol intake, as well as combinations of these factors, with risk of colorectal cancer (CRC).
Methods: We assessed diet using a 62-item food-frequency questionnaire among 45,264 women in the Breast Cancer Detection Demonstration Project (BCDDP) Follow-up Study. After an average of 8.5 years of follow-up, 490 cases of CRC were identified.
Results: Dietary folate showed only a slight inverse association with the risk of CRC (RR = 0.86, 95% CI 0.65–1.13 for high *vs.* low quintile, p for trend = 0.14), and the association for total folate was null. Consuming more than two servings of alcohol per day was only slightly associated with CRC in this cohort (RR = 1.16, 95% CI 0.63–2.14). Combinations of high alcohol and low total folate did not result in a higher risk of CRC. There was no association between methionine and colorectal cancer.
Conclusions: This study shows limited association between alcohol intake and CRC. The non-association of total folate and methionine with CRC, and the null results from the combined folate and alcohol analyses, suggest that what effect alcohol may have on CRC is unrelated to the methyl-group metabolism pathway.

Introduction

Colorectal cancer is the third most common cancer among women in the United States after lung and breast cancer (excluding non-melanoma skin cancer). Strong evidence points to dietary behaviors as among the most important lifestyle factors associated with the risk of colorectal cancer [1]. Several cohort and numerous case–control studies have demonstrated an association between alcohol and colorectal cancer with risk estimates often exceeding 2.0 for comparisons of high *vs.* low consumption groups [1–18]. These and other studies have also provided some indication, though not completely consistent, of an inverse association between folate consumption or blood levels of folate and colorectal cancer [5–7, 10, 11, 14, 16, 19–21]. Studies of colorectal adenomas have shown similar results, *i.e.* an increased risk associated with higher alcohol consumption and a suggestion of decreased risk associated with higher folate consumption [22–29]. Furthermore, Giovannucci *et al.* provided evidence that methionine, found commonly in red meat, is important in the prevention of colon cancer in men consuming more than 20 g of alcohol per day [6]. Although epidemiologic studies generally support these hypotheses (especially for alcohol), not all results are consistent. Some investigators observe little evidence of an association between alcohol and colorectal cancer [1, 30–36], and others observe little evidence of an inverse association for folate [6, 37, 38].

Folate, alcohol, and methionine are all components of complex pathways related to DNA methylation and DNA synthesis. The 5-methyltetrahydrofolate form of folate is the methyl donor in the conversion of homocysteine to methionine, methionine in turn is converted to *S*-adenosyl methionine (SAM), and the methyl group from SAM is transferred to cytosine residues on DNA.

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Methylation of DNA is a basic element of regulating gene expression in the cells. Hypomethylation may increase the risk of colorectal cancer via loss of control of proto-oncogene activity, and folate-deficient diets have been directly associated with hypomethylation status [39]. Alternatively, hypermethylation has been implicated in the inactivation of the von Hippel-Lindau (VHL) and retinoblastoma (Rb) tumor-suppressor genes [40], and in cancer generally [41].

Folate may also mediate carcinogenesis through a second pathway. The 5,10-methylene tetrahydrofolate form of folate is the methyl donor in the conversion of dUMP to dTMP. Failure to synthesize dTMP will result in a deficiency of this nucleotide and will in turn lead to inappropriate incorporation of uracil into the DNA in place of thymidine, a result that has been linked to DNA strand breaks. A break in the p53 tumor-suppressor gene has been linked to an increased risk of colorectal cancer [42].

Alcohol may participate in these methylation pathways by promoting the degradation, inhibiting the absorption, and increasing the excretion of folate [42].

Based on all of the above, Giovannucci *et al.* hypothesized that low-methyl diets, *i.e.* those low in folate and methionine and high in alcohol, would increase the risk of colon cancer. When comparing men consuming a ''low-methyl diet'' to men consuming a ''high-methyl diet'' in the Health Professionals Follow-up Study, Giovannucci *et al.* observed a RR of 2.34 (95% CI 1.28–4.30), thus providing support for the low-methyl diet hypothesis [6]. Also consistent with the methyl-group hypothesis, Giovannucci *et al.* found an almost 40% reduction in risk for colon cancer in the Nurses' Health Study among women who consumed more than 400 μg of folate per day and at least 1.8 g of methionine per day compared to women who consumed $\leqslant 200$ μg of folate per day and less than 1.8 g of methionine [7]. Recently, Su and Arab found a similar reduction in risk among the high-folate, high-methionine, and low-alcohol group in the NHANES I follow-up study, but only among men [21].

The purpose of our study was to test this ''low-methyl diet'' hypothesis and further examine the relationship between folate, alcohol, and methionine and risk of colorectal cancer in a large cohort of women.

Materials and methods

Study population

Between 1973 and 1980 the National Cancer Institute (NCI) and the American Cancer Society sponsored the Breast Cancer Detection Demonstration Project (BCDDP), a breast cancer screening program providing breast examinations to 283,222 women at 29 screening centers in 27 US cities. In 1979 the NCI established a follow-up cohort from a subset of the women who had participated in the BCDDP. The follow-up cohort included all 4275 women from the screening program who had been diagnosed with breast cancer, all 25,114 women who had biopsies indicating benign breast disease, and all 9628 who had been recommended for biopsy or breast surgery but who did not have a surgical procedure. An additional 25,165 women who neither received nor were recommended for biopsy were matched with the above-listed subjects on age, time of entry into the screening program, ethnicity, screening center, and length of participation in the BCDDP for a total of 64,182 women selected for entry into the follow-up cohort.

Between 1979 and 1981 a total of 61,433 (96%) of the women in the follow-up cohort completed a baseline questionnaire and were therefore eligible for further participation in the study. Between 1987 and 1989 the participants received a mailed questionnaire that included a 62-item food-frequency questionnaire (FFQ) in addition to questions relating to smoking, alcohol use, height, weight, dietary supplement use, physical activity, and lifetime history of colorectal cancer. Between 1993 and 1995 the participants received a follow-up questionnaire that updated previously reported, non-dietary information, obtained information on NSAID use, and allowed for self-reports of incident colorectal cancer and other medical conditions. Between 1995 and 1998 the participants received a final follow-up questionnaire on which they could report incident cases of colorectal cancer and other medical conditions as well as updating previous information. Non-responders to these questionnaires received active follow-up including repeated phone calls and mailings.

We excluded from our analytic cohort 9740 women who did not complete a 1987–1989 questionnaire since it contained the dietary assessment instrument. Of the 9740 women without a 1987–1989 questionnaire 3066 had died, 505 did not complete the questionnaire due to illness, 1459 refused, and 4710 were either non-responsive or unable to be contacted. In addition to these, we excluded, in order, 479 women with a colorectal cancer reported on their 1987–1989 or prior questionnaires, six women whose reported entry date occurred on or after their exit date (see definition of exit dates below), and 5647 women who skipped more than 30 items on their FFQ or who had a reported total energy intake above 3800 or below 400 kcal per day. For this study, women with implausible or unusually high intakes of folate

(>2400 μg per day, n = 172) or alcohol (>6 servings per day, n = 125) were also excluded, leaving 45,264 women in the final analytic cohort.

Cohort follow-up and case ascertainment

Follow-up for these analyses began at the date of completion of the 1987–1989 questionnaire. The maximum follow-up period for each participant extended until the date of completion for the 1995–1998 follow-up questionnaire, the last contact in the 1995–1998 follow-up period if no questionnaire was completed, or the "end of study date" for those not contacted in the 1995–1998 follow-up period. "End of study date" is the estimated date on which subjects would have completed the 1995–1998 questionnaire (using mean time intervals between questionnaires from the rest of the cohort) had they actually completed one.

We defined exit date for each subject as the earliest among: date of diagnosis for colorectal cancer, date of death from cause other than colorectal cancer, date of 1995–1998 questionnaire, or "end of study date" if the subject did not complete a 1995–1998 questionnaire.

In the final analytic cohort, 90% (40,865 women) had complete follow-up through 1995–1998, meaning their exit date corresponded to either the date of their first colorectal cancer diagnosis, the date they filled out the 1995–1998 questionnaire, or their date of death from a cause other than colorectal cancer. Of the remaining 10%, 1224 women were contacted in 1995–1998 but did not fill out a questionnaire; 2651 filled out a 1993–1995 questionnaire but were not contacted in 1995–1998; and 524 filled out neither a 1993–1995 nor a 1995–1998 questionnaire.

We defined cases to be all invasive carcinomas of the colon or rectum, ICD-0 site codes 153.0–153.4, 153.6–153.9, and 154.0–154.1. Case ascertainment came first through self-reports of colorectal cancer from the 1993–1995 and 1995–1998 questionnaires. We obtained pathology reports for 247 (80%) of the 313 women who provided self-reports of a diagnosis of colorectal cancer. Of the 247 self-reported cases with medical records the pathology reports confirmed 232 (94%). In light of this high confirmation rate for the self-reports with medical records, we classified as cases the remaining 66 self-reports of colorectal cancer without pathology reports. Exclusion of these 66 cases did not materially affect the results (data not shown). Women with pathology reports contradicting self-reported colorectal cancers were not included as cases. Pathology reports obtained for self-reported conditions unrelated to colorectal cancer identified 17 more cases of colorectal cancer. A search of the National Death Index (through 1997) identified an additional 108 individuals with death certificates indicating a diagnosis of colorectal cancer. Finally, we used last-known place of residence for each subject to match against state cancer registries for those states whose registries consented to participate in the study. Subjects sent to match against state registries (73.5% of the analytic cohort) did not differ in any material way with respect to distribution of risk factors from those we did not send to match. This procedure resulted in the identification of a further 67 colorectal cancer cases. Thus the total number of cases in the analytic cohort over the follow-up period was 490.

Dietary assessment

As part of the 1987–1989 follow-up questionnaire, respondents completed a 62-item Block/NCI FFQ to assess usual dietary and alcohol intake over the previous year. Detailed descriptions of this FFQ and its validity have appeared elsewhere [43–45]. Software designed for this FFQ yielded estimates of daily intakes for total energy and micronutrients [45].

For these analyses the intakes from fruits, vegetables, red meat, and grains were expressed in terms of estimated daily frequency of consumption per 1000 kcal of total energy per day. Similarly, standard units of nutrient (*e.g.* milligrams) per 1000 kcal were used for alcohol, dietary folate, calcium, and vitamin D in all analyses.

Statistical analysis

We used Cox proportional hazards regression (PROC PHREG in SAS version 6.12) with age as the underlying time metric to generate rate ratios (RRs) and 95% confidence intervals (CIs) for dietary folate, total folate (dietary folate plus supplemental folate), methionine, and alcohol both separately and in combination (proportional hazards assumptions were met). All *p*-values were two-sided. Trend tests assessed the significance of the parameter estimate for a change in the ordinal value of quintile indicator variables.

We adjusted dietary folate and methionine for total energy intake using the multivariate nutrient density method (nutrient per 1000 kcal per day with total energy in the model). For total folate we did not energy-adjust the intake of folate from supplements since intake of a nutrient from supplement use is not fundamentally related to energy intake as would be a nutrient derived from food. Thus, in order to arrive at a single value for total folate, we added the energy-adjusted dietary folate (the residual of dietary folate regressed on energy plus the mean value for dietary folate in the analytic cohort)

to the intake of folate from supplements. We adjusted alcohol for total energy using the standard method (*i.e.* we included total calories as a covariate in the model). Using alternative energy adjustment methods for any of the analyses did not materially affect the final results.

In considering potential confounders we tested candidates by entering the risk factors individually into each of the four energy-adjusted models (*i.e.* dietary folate, total folate, methionine, and alcohol). We judged a change of greater than 10% in the parameter estimate from the energy-adjusted model as evidence for confounding, and we included these covariates in all future "fully adjusted" models. We tested the following variables in this manner: NSAIDs (non-steroidal anti-inflammatory drugs) use (yes/no), smoking (ever/never), education (through high school/beyond high school), BMI (kg/m^2), height, weekday physical activity index expressed in units of Metabolic Equivalent Time [46], vitamin D, calcium, total fat, fiber, red meat, and grains. NSAIDs included aspirin, ibuprofen (Advil, Motrin, Nuprin), Naprosyn, and other pain-relieving drugs, but excluded Tylenol. We considered women users of NSAIDs if they had used these drugs at least once a week for at least one year. Among these potential confounders total fat emerged as important in the total folate model, calcium and vitamin D were important in the methionine model, and smoking was important in the alcohol model. No other variables met the criteria established above in any other models.

For each statistical analysis we tested a model controlling for energy as well as the other main variables of interest (*e.g.* dietary folate models controlled for methionine and alcohol) and any important confounder we identified in the manner described above. We also tested a model with all potential confounders included regardless of their individual importance in the confounder tests described above to see if the combined effect of these variables as a group was important. In no case, however, did rate ratios from these larger models differ materially from the models adjusting for the more limited set of covariates (data not shown); therefore we do not present them in the results section below.

To test whether the effects of one variable depended on the level of others, we analyzed models with interaction terms using both continuous and categorical measures of folate, alcohol, and methionine. We also examined models with interaction terms for folate, methionine, and alcohol with a categorical measure of NSAIDs use (ever/never). We compared the -2 log-likelihood statistics for models with and without both additive and multiplicative interaction terms to test the significance of any interaction effects considering *p*-values of less than 0.05 as evidence of interaction.

Results

Women entered the analytic cohort at an average age of 61.9 years (range: 40–93 years), and mean follow-up time in the study was 8.5 years. Baseline characteristics of the participants according to quintile of dietary folate and category of alcohol consumption appear in Table 1a and Table 1b, respectively. Age, supplemental folate, and consumption of fruits and vegetables increased steadily across quintiles of dietary folate, whereas the percentage of women who smoked, total energy intake, total fat, and consumption of red meat and alcohol steadily decreased across quintiles. Smoking, use of NSAIDs, the proportion of women with more than a high school education, and total energy intake increased consistently across categories of alcohol consumption, while BMI decreased across categories of alcohol.

Table 2 presents results from separate assessments of the relationships between folate, methionine, and alcohol intake and colorectal cancer. Women in the highest quintile of dietary folate had a slightly reduced risk of developing colorectal cancer compared to women in the lowest quintile (RR = 0.86, 95% CI 0.65–1.13). By contrast, there was no association between intake of total folate and colorectal cancer comparing the highest quintile to the lowest quintile (RR = 0.94, 95% CI 0.70–1.26). Similarly, we saw no relationship between colorectal cancer and quintiles of methionine when comparing high to low quintiles of intake (RR = 0.93, 95% CI 0.66–1.30).

We observed a very slight, non-significant increase in risk among women who consumed more than two drinks of alcohol per day *versus* nondrinkers (RR = 1.16). With only 11 cases in the top category of consumption, the confidence intervals were wide (95% CI 0.63–2.14), and the test for trend was not significant ($p = 0.84$).

In a combined analysis of folate and methionine (Table 3), women in the highest quintile of dietary folate who also consumed 90 g/1000 kcal per day or greater of methionine had a reduced risk compared to women in the low-folate–low-methionine category (RR = 0.57, 95% CI 0.32–1.02). Comparisons of the -2 log-likelihood ratios in models with and without interaction terms for folate and methionine, however, gave no indication of an interaction effect (data not shown). For total folate, again, there was no indication of any association with colorectal cancer even after stratifying on methionine.

We used a 3×3 categorical approach to examine the combined relationship of alcohol and dietary folate or alcohol and total folate with colorectal cancer. For dietary folate (Table 4), in no case did women who

Table 1a. Baseline characteristics of 45,264 women in the BCDDP cohort study according to quintile of dietary folate intake

	Quintile of dietary folate				
	1	2	3	4	5
Dietary folate (μg/1000 kcal)	114	160.4	196	241	367
Supplemental folate (μg)	133	141	152	164	162
Total folate (μg)[a]	270	334	388	449	594
Dietary alcohol (g)	5.08	4.33	3.74	3.12	2.56
Dietary methionine (g/1000 kcal)	0.70	0.75	0.77	0.78	0.77
Age	60.5	61.3	61.9	62.2	63.4
Total energy intake (kcal)	1407	1338	1261	1184	1190
BMI (kg/m^2)	25.0	24.9	24.8	24.5	24.2
Height (inches)	63.9	64.0	64.0	64.0	63.9
Physical activity index (METs)	56.2	56.8	57.0	57.2	57.5
Vegetables (servings/1000 kcal)	1.69	2.20	2.46	2.78	3.15
Fruit (servings/1000 kcal)	0.54	0.86	1.10	1.30	1.63
Red meat (g/1000 kcal)	34.0	30.3	27.1	23.5	19.6
Total fat (%)	40.9	37.7	35.0	32.6	28.7
NSAID users (%)	37.5	39.5	39.1	39.7	37.9
Smokers (current and former) (%)	47.4	43.3	41.8	41.8	40.9
Greater than high school education (%)	39.9	45.2	47.7	49.8	48.2

[a] Energy adjusted as described in text.

Table 1b. Baseline characteristics of 45,264 women in the BCDDP cohort study according to category of alcohol intake

	Category of alcohol intake (drinks per day)				
	0	0.01–0.50	0.51–1.00	1.01–2.00	> 2.00
No.	26,776	10,475	4,669	2,438	906
Dietary folate (μg/1000 kcal)	219	218	211	195	168
Supplemental folate (μg)	142	160	166	169	152
Total folate (μg)[a]	400	420	422	411	360
Dietary alcohol (g)	0.0	2.7	11.0	20.8	43.4
Dietary methionine (g/1000 kcal)	0.76	0.77	0.73	0.69	0.62
Age	62.6	60.6	61.3	60.7	60.1
Total energy intake (kcal)	1246	1277	1324	1386	1596
BMI (kg/m^2)	25.2	24.3	23.5	23.3	23.5
Height (inches)	63.8	64.1	64.3	64.4	64.5
Physical activity index (METs)	57.1	56.8	56.6	56.3	55.8
Vegetables (servings/1000 kcal)	2.45	2.52	2.51	2.37	2.11
Fruit (servings/1000 kcal)	1.11	1.10	1.04	0.98	0.77
Red meat (g/1000 kcal)	27.9	26.1	25.1	24.3	23.5
Total fat (%)	35.3	35.5	34.4	32.9	29.8
NSAID users (%)	37.4	40.3	40.5	41.8	43.1
Smokers (current and former) (%)	33.6	50.0	63.0	67.8	72.1
Greater than high school education (%)	39.0	53.1	58.9	64.5	63.4

[a] Energy adjusted as described in text.

consumed higher alcohol or lower folate have an increased risk over the reference group (non-drinkers with greater than 232 μg/1000 kcal per day of folate). In a similar 3×3 analysis examining total folate instead of dietary folate (Table 4), no combination of folate and alcohol consumption showed any significant increase in risk compared to the reference category. For both dietary and total folate there was no greater risk among women in the low-folate *vs*. the high-folate category within the high-alcohol category.

Table 2. Relative risk of colorectal cancer by quintile of dietary folate, total folate, and methionine, and category of alcohol intake

	Quintile/category					Trend
	1	2	3	4	5	
Dietary folate						
μg/1000 kcal	<142	142–177	178–215	216–272	>272	
Energy-adjusted RR (95% CI)	1.00 (ref.)	0.78 (0.59–1.05)	0.89 (0.67–1.18)	0.98 (0.74–1.29)	0.84 (0.64–1.12)	$p = 0.15$
Fully adjusted[a] RR (95% CI)	1.00 (ref.)	0.79 (0.59–1.06)	0.90 (0.68–1.19)	0.99 (0.75–1.31)	0.86 (0.65–1.13)	$p = 0.14$
Total folate						
μg[b]	<188	188–253	254–374	375–633	>633	
Energy-adjusted RR (95% CI)	1.00 (ref.)	0.94 (0.70–1.25)	0.90 (0.67–1.19)	0.98 (0.74–1.30)	1.02 (0.77–1.35)	$p = 0.63$
Fully adjusted[c] RR (95% CI)	1.00 (ref.)	0.94 (0.70–1.25)	0.89 (0.66–1.20)	0.97 (0.73–1.30)	1.01 (0.75–1.35)	$p = 0.67$
Methionine						
g/1000 kcal	<0.58	0.58–0.67	0.68–0.77	0.78–0.91	>0.91	
Energy-adjusted RR (95% CI)	1.00 (ref.)	0.96 (0.73–1.26)	1.01 (0.77–1.32)	0.96 (0.72–1.26)	0.85 (0.64–1.14)	$p = 0.33$
Fully adjusted[d] RR (95% CI)	1.00 (ref.)	0.98 (0.74–1.29)	1.04 (0.79–1.39)	1.00 (0.74–1.35)	0.93 (0.66–1.30)	$p = 0.83$
Alcohol						
Servings/day	0	0.01–0.50	0.51–1.00	1.01–2.00	>2.00	
Cases/subjects	301/26,776	101/10,475	52/4669	25/2438	11/906	
Energy-adjusted RR (95% CI)	1.00 (ref.)	0.95 (0.76–1.20)	1.07 (0.80–1.44)	1.01 (0.67–1.53)	1.26 (0.69–2.31)	$p = 0.61$
Fully-adjusted[e] RR (95% CI)	1.00 (ref.)	0.92 (0.73–1.16)	1.00 (0.74–1.35)	0.94 (0.62–1.42)	1.16 (0.63–2.14)	$p = 0.84$

[a] Adjusted for energy, methionine, and alcohol.
[b] Dietary portion of total folate is energy-adjusted as described in the text.
[c] Adjusted for energy, methionine, alcohol, and total fat.
[d] Adjusted for energy, dietary folate, alcohol, calcium, and vitamin D.
[e] Adjusted for energy, dietary folate, methionine, and smoking.

Table 3. Relative risk of colorectal cancer by quintile of dietary or total folate stratified by methionine level

Methionine level	Quintile of folate				
	1	2	3	4	5
Dietary folate (μg/1000 kcal)					
<0.90 g/1000 kcal	<142	142–177	178–215	216–272	>272
Cases/participants	86/7777	64/7331	81/7033	86/6868	86/6902
RR[a] (95% CI)	1.00 (ref.)	0.74 (0.53–1.02)	0.95 (0.70–1.29)	1.01 (0.75–1.38)	0.94 (0.69–1.29)
≥0.90 g/1000 kcal					
Cases/participants	14/1276	19/1722	16/2021	23/2184	15/2150
RR[a] (95% CI)	1.00 (0.56–1.77)	1.00 (0.60–1.67)	0.70 (0.40–1.21)	0.91 (0.55–1.48)	0.57 (0.32–1.02)
Total folate (μg[b])					
<0.90 g/1000 kcal	<188	188–253	254–374	375–633	>633
Cases/participants	77/7624	74/7195	77/6970	83/7232	92/6890
RR[c] (95% CI)	1.00 (ref.)	0.94 (0.68–1.30)	0.95 (0.69–1.32)	1.01 (0.74–1.39)	1.12 (0.81–1.54)
≥0.90 g/1000 kcal					
Cases/participants	16/1429	19/1858	17/2083	18/1821	17/2162
RR[c] (95% CI)	1.14 (0.66–1.98)	1.01 (0.60–1.69)	0.75 (0.43–1.30)	0.92 (0.53–1.57)	0.72 (0.41–1.25)

[a] Adjusted for energy, alcohol, calcium, and vitamin D.
[b] Dietary portion of total folate is energy-adjusted as described in the text.
[c] Adjusted for energy, alcohol, calcium, vitamin D, and total fat.

As regular NSAID use has been associated with a reduced risk of colon cancer [1], we performed a stratified analysis to examine the associations between these drugs and the relationships among folate and methionine and colorectal cancer. Among nonusers, but not among users of NSAIDs, we observed a slight reduction in risk for the highest quintile of dietary folate intake (RR = 0.82, 95% CI 0.59–1.13); but for total folate there was no association with colorectal cancer, regardless of NSAID use (data not shown). Likewise, we

Table 4. Relative risk of colorectal cancer, level of alcohol, and tertiles of dietary and total folate. Folate intake adjusted for energy using nutrient density method

	Alcohol, servings/day		
	0	0.01–1.00	>1.00
Dietary folate			
High (>232 μg/1000 kcal)			
Cases/participants	118/9356	46/5016	6/716
RR[a] (95% CI)	1.00 (ref.)	0.77 (0.55–1.09)	0.69 (0.30–1.58)
Medium (167–232 μg/1000 kcal)			
Cases/participants	88/8596	64/5373	11/1119
RR[a] (95% CI)	0.88 (0.67–1.16)	1.06 (0.78–1.45)	0.87 (0.47–1.62)
Low (<167 μg/1000 kcal)			
Cases/participants	95/8824	43/4755	19/1509
RR[a] (95% CI)	0.97 (0.74–1.28)	0.85 (0.59–1.21)	1.10 (0.67–1.80)
Total folate			
High (>514 μg[b])			
Cases/participants	104/8427	66/5456	11/1205
RR[c] (95% CI)	1.00 (ref.)	1.03 (0.75–1.40)	0.76 (0.41–1.43)
Medium (231–514 μg[b])			
Cases/participants	97/9121	50/5061	11/906
RR[c] (95% CI)	0.87 (0.66–1.14)	0.86 (0.61–1.21)	1.04 (0.56–1.96)
Low (<230 μg[b])			
Cases/participants	100/9228	37/4627	14/1233
RR[c] (95% CI)	0.99 (0.75–1.31)	0.76 (0.52–1.11)	0.98 (0.56–1.73)

[a] Adjusted for energy, methionine, and smoking.
[b] Dietary portion of total folate is energy-adjusted as described in the text.
[c] Adjusted for energy, methionine, total fat, and smoking.

did not observe any evidence of an association between methionine and risk of colorectal cancer after stratifying on use of NSAIDs (data not shown).

When we stratified alcohol consumption on NSAID use (Table 5), there was a modest, non-significant association between alcohol and colorectal cancer among nonusers of NSAIDs. The RR among nonusers consuming more than two alcoholic drinks per day compared to nondrinkers was 1.39 (95% CI 0.71–2.75). Among users of NSAIDs there was no evidence of an association between alcohol consumption and risk of colorectal cancer. Addition of interaction terms combining NSAIDs and categories of folate, alcohol, or methionine did not significantly improve the fit of the

Table 5. Relative risk of colorectal cancer by alcohol category stratified by use of NSAIDs and smoking

	Servings of alcohol per day					Trend
	0	0.01–0.50	0.51–1.00	1.01–2.00	>2.0	
Nonusers of NSAIDs						
Cases/participants	228/16,753	65/6259	35/2778	17/1419	9/516	
RR[a] (95% CI)	1.00 (ref.)	0.83 (0.63–1.10)	0.96 (0.67–1.38)	0.95 (0.58–1.57)	1.39 (0.71–2.75)	$p = 0.48$
Users of NSAIDs						
Cases/participants	73/10,023	36/4216	17/1891	8/1019	2/390	
RR[a] (95% CI)	1.00 (ref.)	1.21 (0.81–1.82)	1.15 (0.67–1.98)	0.98 (0.47–2.07)	0.69 (0.17–2.86)	$p = 0.72$
Nonsmokers						
Cases/participants	187/17,773	47/5240	22/1728	8/786	4/253	
RR[b] (95% CI)	1.00 (ref.)	0.96 (0.69–1.32)	1.33 (0.86–2.08)	1.07 (0.53–2.18)	1.78 (0.66–4.83)	$p = 0.57$
Smokers						
Cases/participants	114/9003	54/5235	30/2941	17/1652	7/653	
RR[b] (95% CI)	1.00 (ref.)	0.86 (0.62–1.19)	0.80 (0.54–1.20)	0.83 (0.50–1.39)	0.89 (0.41–1.93)	$p = 0.83$

[a] Adjusted for energy, dietary folate, methionine, and smoking.
[b] Adjusted for energy, dietary folate, and methionine.

models. Finally, we excluded NSAID users from the 3×3 analyses of folate and alcohol and saw no material change in the results (data not shown).

We also performed a stratified analysis of alcohol consumption and colorectal cancer by smoking status (Table 5). The excess risk associated with alcohol was present only among nonsmokers: the RR comparing women who consumed more than two drinks per day to nondrinkers was 1.78 (95% CI 0.66–4.83). Among smokers (former and current) there was no association between alcohol consumption and colorectal cancer (RR = 0.89, 95% CI 0.41–1.93 for greater than two drinks per day *vs.* abstainers).

The results presented above were quite stable and did not vary substantially after stratifying by grade of colorectal cancer diagnosis or excluding women whose diagnosis of colorectal cancer was based solely on a death certificate report. Excluding women with a prior history of breast disease did not materially affect our results, nor did restricting the analyses to women who neither received nor were recommended for biopsy. Similarly, excluding women with a diagnosis of colorectal cancer within the first 2 years of follow-up did not change the results in any qualitative sense. Including women with greater than 2400 μg per day of folate or six servings of alcohol per day in the analyses did not materially alter the results.

Discussion

In our analysis of the main effect of folate we observed a very modest inverse association between dietary folate and colorectal cancer. For the highest quintile of intake the RR was 0.86 (95% CI 0.65–1.13) compared to the low-folate quintile. The linear trend test, however, was not significant ($p = 0.14$). Perhaps of more consequence, when looking at total folate (dietary folate plus folate from supplements), we observed no evidence of any relationship with colorectal cancer. In combination these results suggest that, if there was any association between dietary folate and colorectal cancer in this cohort, it was not due to folic acid *per se* but rather to confounding by some other factor(s) correlated with dietary folate.

We have not been able to identify what these factors might be. We tested all strongly suspected risk factors for colorectal cancer as potential confounders, and none was important in the dietary folate analyses. In a separate set of analyses we determined that neither fruits nor vegetables had any significant association with colorectal cancer in this cohort and therefore were not candidates to be confounders [47]. Thus we must consider residual confounding as a possible explanation for these results. An alternative explanation is that the inverse association in the fifth quintile of dietary folate may simply be a chance result.

One potential qualification to these observations relates to the possible time lag between use of nutrient supplements and reduction in risk from disease. Giovannucci *et al.* have reported findings in which only very long-term use (14 or more years) of supplements resulted in lower risk of colorectal cancer [7]. This observation would suggest that the etiologically important period for folate is long before the actual onset of disease. While diet may show some consistency over time, supplement use is much more variable, which means recent assessment of nutrient intakes from supplements may not be a useful proxy for long-term use. In the BCDDP cohort we have no information concerning duration of use for supplements; thus, if we accept that only supplement use 15-plus years prior to onset is important, the possibility of misclassification for folate in the etiologically relevant period does exist.

These findings should be considered with the main body of epidemiologic literature which shows some evidence of an inverse association between colorectal cancer/neoplasia and folate. This evidence, though, is by no means strong or consistent. In most earlier studies the associations are either null [6, 37, 38], exist only in differing subsets of the study population or for differing subsites [5, 10, 11, 14, 16, 20, 21, 23], are attenuated substantially after adjustment for confounders [11, 22], or, as in the results presented above, exist for dietary but not total folate [22]. Only two studies presented results compatible with a general reduction in risk with increasing intake of folate [7, 26].

We observed no association in this study population between colorectal cancer and dietary methionine. In contrast to earlier reports [6, 26], this null association remained even after stratification on either alcohol or NSAIDs (data not shown). While the association between methionine and colorectal cancer does appear to be null in the BCDDP cohort, the level of consumption for this amino acid, unlike folate, was not high. In the Health Professionals Follow-up Study (HPFS) cohort [6], the top quintile of intake reported greater than 2.44 g of methionine per day (in a study population consuming roughly 1850 kcal per day on average), but in the top quintile of the BCDDP cohort subjects consumed as few as 0.91 g/1000 kcal per day. The HPFS cohort consisted entirely of men, a group that typically eats more meat (the major dietary source of methionine) compared to women, and used a more extensive FFQ providing the likelihood of more complete nutritional assessment, so it is not surprising to observe higher

methionine intakes in that cohort. Even so, if there is a minimum threshold at which methionine begins to have an effect, the women in the BCDDP cohort, even those in the top quintile, may simply have consumed too little for us to observe a reduction in risk. Furthermore, low between-person variation in methionine intake in the BCDDP cohort (0.61–0.87 g/1000 kcal for 25th–75th percentile) placed a large burden on the FFQ in terms of its ability to categorize individuals properly for this nutrient. The 60-item FFQ we used may have limited precision in doing so. In these respects the current null findings may not provide a rigorous challenge to earlier reports indicating a relationship between methionine and colorectal cancer; nonetheless they fail to provide additional support for those results.

Even if the individual nutrients studied in isolation were not strongly associated with disease, it remained possible that, as the methyl group metabolism hypothesis would suggest, the effect of one might depend on the level of the others. Therefore, we explored this hypothesis further by looking at combined effects of methionine and folate in stratified analyses. The results did provide some support to the hypothesis that a "low-methyl" diet would increase risk, as women in the high-folate, high-methionine category did have a decreased risk compared to the reference group. This reduction in risk was greater than what we observed in the high quintile of the low-methionine strata, but there was substantial overlap in the 95% CIs between these two rate ratios. Furthermore, the overall pattern of RRs in this analysis lacked consistency, thus diminishing our confidence that increasing deficiencies in both of these nutrients simultaneously would result in ever-increasing risk of colorectal cancer. The results for total folate were null, further suggesting that folic acid *per se*, or folic acid and methionine-mediated methyl-group metabolism, does not contribute significantly to a modification in risk of colorectal cancer. Once again, however, the level of methionine consumption in this cohort was not high, potentially limiting our ability to see effects of widely differing intakes.

The 3×3 categorical analyses of alcohol and folate did not offer any additional evidence to suggest that methyl-group compromised individuals would be at increased risk. Taken together these analyses gave no suggestion of a modification in risk for alcohol as a result of decreasing intake of folate. However, small numbers of cases in the high-alcohol–low-folate groups yielded wide confidence intervals, making these results somewhat difficult to interpret.

Analyses of alcohol intake provided little evidence of an association with colorectal cancer. The elevation in risk we observed was only modest, and appeared only in the top category of intake when looking at alcohol individually. Stratification on smoking status, however, provided evidence that an alcohol association might exist among women who did not smoke. Thus while alcohol increased the risk of colorectal cancer among nonsmokers (even at the low levels of intake we observe in this cohort), no additional risk was conferred to women who were smokers as a result of consuming two drinks per day.

It is important to note that the range of consumption for alcohol in the BCDDP cohort was quite low. Of the 45,264 women in the cohort only 3344 (7%) reported having more than 1.0 drink per day. Over half the cohort (26,636 women) reported not consuming any alcohol. Given this modest level of consumption the modest associations we observed between alcohol and colorectal cancer were potentially noteworthy. With higher levels of consumption the risk of colorectal cancer could conceivably be substantially higher than reported here. Moreover, it is possible that such high levels of consumption could result in elevated risks for colorectal cancer even among smokers (*i.e.* those who might otherwise have maximized their risk). Earlier studies provide some evidence of increased risk with consumption levels above one drink per day. The great majority of studies that afforded such analysis gave at least some indication of additional risk in categories beyond one drink per day [1, 6, 8, 11, 12, 18, 23, 26, 28, 32]. Of the remaining such studies showing some increase in risk overall, only six showed no additional risk with consumption over one drink per day [3, 13, 15, 24, 25, 27].

In assessing the "low-methyl" *vs.* "high-methyl" diet hypothesis we saw no evidence of an association between colorectal cancer and the "low-methyl" diet. Together with the contrasting dietary *vs.* total folate results and the null methionine results in the main-effects analyses, these results suggest that what modest association of alcohol we observed with colorectal cancer risk was unrelated to folate in the BCDDP follow-up study. As such, data from this cohort fail to provide support for the hypothesis that methyl-group metabolism, as influenced by the availability of folate in the diet, plays an important role in determining risk of colorectal cancer. Others have discussed the limitations facing epidemiologic studies relying on FFQs for dietary assessment in similar populations [47–49], and this study was not free of them. These limitations (including lack of range and measurement error in exposure variables) frequently result in the attenuation of risk estimates; consequently, we could not exclude the possibility that individuals with a greater degree of "methyl deficiency" would truly be at higher risk. These results provided

only limited evidence to implicate alcohol as a potentially important risk factor for colorectal cancer; moreover, the findings suggest that, if alcohol does increase risk, it does so through a pathway unrelated to methyl-group metabolism.

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